Planta 136, 77–89 (1977)

Planta

Distribution and Structure of the Plasmodesmata in Mesophyll and Bundle-sheath Cells of *Zea mays* L.

R. F Evert, W. Eschrich, and W. Heyser

Department of Botany, University of Wisconsin, Madison, WI 53706, USA and Forstbotanisches Institut der Universität Göttingen, Büsgenweg 2, D-3400 Göttingen-Weende, Federal Republic of Germany

Abstract. In leaf blades of *Zea mays* L. plasmodesmata between mesophyll cells are aggregated in numerous thickened portions of the walls. The plasmodesmata are unbranched and all are characterized by the presence of electron-dense structures, called sphincters by us, near both ends of the plasmodesmatal canal. The sphincters surround the desmotubule and occlude the cytoplasmic annulus where they occur. Plasmodesmata between mesophyll and bundle-sheath cells are aggregated in primary pit-fields and are constricted by a wide suberin lamella on the sheath-cell side of the wall. Each plasmodesma contains a sphincter on the mesophyll-cell side of the wall. The outer tangential and radial walls of the sheath cells exhibit a continuous suberin lamella. However, on the inner tangential wall only the sites of plasmodesmatal aggregates are consistently suberized. Apparently the movement of photosynthetic intermediates between mesophyll and sheath cells is restricted largely or entirely to the plasmodesmata (symplastic pathway) and transpirational water movement to the cell walls (apoplastic pathway).

Key words: Apoplast – Bundle sheath – Mesophyll – Plasmodesmata – Suberin lamella – Symplast – *Zea*.

Introduction

Interest in the mechanisms and pathways for the short-distance transfer of substances in plants – especially in leaves – has increased significantly during the past several years. Much of this interest has been stimulated by studies on metabolic transport in C_4 photosynthesis (Osmond and Smith, 1976) and on phloem loading and transport in the minor veins of leaves (Geiger, 1976). The pathways followed by both the intermediates and products of photosynthesis between leaf tissues and by water and solutes moving from the xylem to other regions of the leaf may be symplastic or apoplastic, or a combination of the two. In order to determine the principal pathways of such substances in the leaf it is essential to know the frequency and distribution of plasmodesmata between the various cell types of the leaf. Unfortunately, only two fairly detailed studies on plasmodesmatal frequency in leaves have been undertaken (Kuo et al., 1974; Olesen, 1975) and these have dealt only with the mesophyll-mestome or mesophyll-bundle-sheath cell interface.

At this time we are reporting on the distribution and structure of the plasmodesmata between epidermal, mesophyll, bundle-sheath, and vascular parenchyma cells in the leaf of *Zea mays* L. In a subsequent article, we will report on similar aspects of the connections between the remaining cell types in small and intermediate veins of the leaf.

The *Z. mays* leaf was selected for study primarily because it has proved highly suitable for experimental studies on phloem transport (Heyser et al.,1975; Heyser et al., 1976). A thorough understanding of all the data obtained from such studies requires a detailed knowledge of the anatomy and cytology of the leaf. Dealing primarily with the mesophyll and bundle sheath cells, the present article contributes to our understanding of the probable pathways followed by photosynthetic intermediates between mesophyll and bundle-sheath cells in C_4 monocotyledons.

Materials and Methods

The tissues used in this study were obtained from corn plants (*Zea mays* L., cv. Prior; Samen-Kröbel, Göttingen, Germany) grown in either the greenhouse (18–25° C; natural daylight during summertime, additional light from mercury vapor lamp, 16 h, dur-

Abbreviation: ER-endoplasmic reticulum

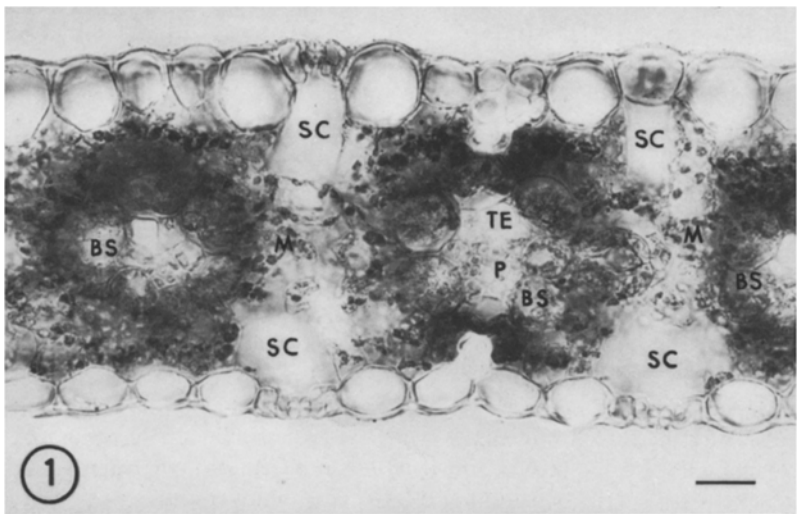
ing wintertime) or growth chambers (25° C; humidity 65%; light 16 h, 3.10^{-7} W. m^{-2}) of the Forstbotanisches Institut, University of Göttingen. Specifically, the tissue was obtained from median portions of the fifth visible leaf, counted from above, of plants ca. 80 cm tall. Some tissues were fixed in 6% glutaraldehyde in cacodylate buffer, pH 7.0, for 6 h at room temperature, and postfixed in 2% osmium tetroxide in cacodylate overnight in a refrigerator; others were fixed solely in osmium tetroxide. Embedment was in either Epon-Araldite or Spurr's epoxy resin. Thin sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed and photographed with a Hitachi HU-11C microscope. Sections 0.5 µm thick and stained with uranyl magnesium acetate and lead citrate, were viewed and photographed at 1 MeV with the High-voltage Electron Microscope at the University of Wisconsin, Madison.

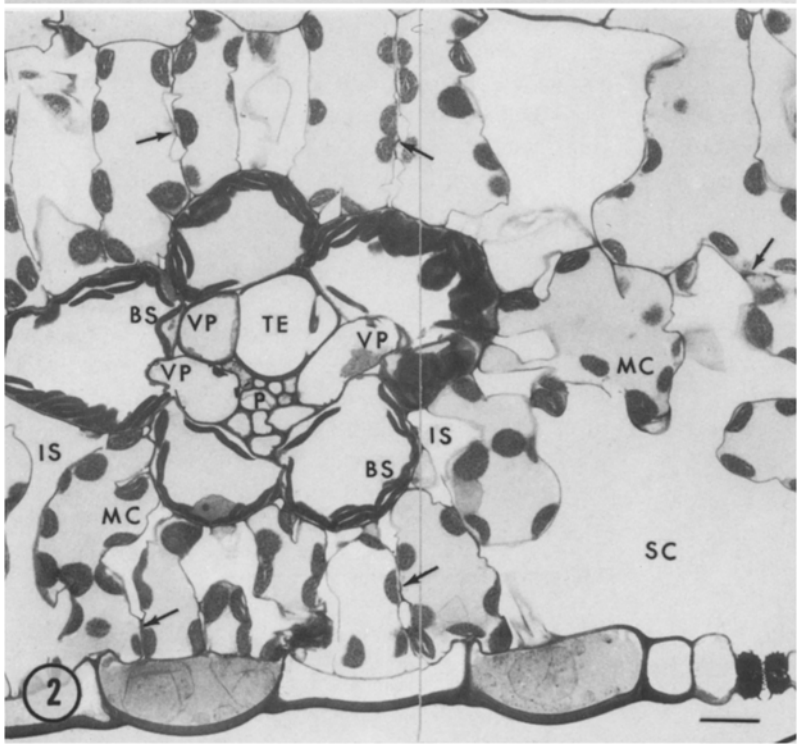
The terminology used for plasmodesmatal structure in this article was adopted from Gunning and Robards (1976b).

Results

Brief Description of the Leaf

In common with the leaves of other grasses, those of *Z. mays* have numerous "parallel" or longitudinal vascular bundles. As noted by Esau (1943), the longitudinal bundles of the blade fall roughly into three groups according to size and structure: large, small, and intermediate. The small bundles are the most numerous, outnumbering the intermediate bundles by about 7:1 and the large bundles by about 10:1. The different-sized bundles alternate fairly regularly with one another and are interconnected by small trans-





Figs. 1 and 2. Transverse sections of portions of leaf blades of *Zea mays*. *BS* bundle sheath; *IS* intercellular space; *M* mesophyll; *MC* mesophyll cell; *P* phloem; *SC* substomatal chamber; *TE* tracheary element; *VP* vascular parenchyma cell

Fig. 1. Photomicrograph of free-hand section showing one intermediate (near center) and two small (right and left) veins. Bar = 4.0 µm

Fig. 2. High-voltage electron micrograph showing small vein and contiguous chlorenchymatous tissues. Arrows point to thickened protions of mesophyll cell walls in which plasmodesmata are aggregated. Bar = 9.75 µm

verse veins. In the leaves examined, the transverse veins comprised about 12% of the total vein length.

The present study was devoted almost entirely to portions of leaves containing small and intermediate veins (Figs. 1, 2). Both groups of veins have chlorenchymatous bundle sheaths with walls somewhat thicker than those of the mesophyll. A hypodermal sclerenchyma strand commonly occurs on both sides of the intermediate bundles between epidermis and sheath (Fig. 1).

The anatomy of the *Zea* leaf is typical of C_4 species, with the mesophyll more or less radially arranged around the chlorenchymatous bundle sheath. Within the sheath cells the chloroplasts are arranged centrifugally (Fig. 2). A single layer of mesophyll cells, described by Metcalfe (1960) as "palisade-like", occurs between epidermis and bundle-sheath cells on both surfaces of the leaf. The resemblance of these cells to palisade cells is however apparent only in transverse sections (Figs. 1, 2). When viewed in either longitudinal sections or macerations (Fig. 3), they can be seen to be deeply lobed, or branched, as are all mesophyll cells of the blade.

Whereas the bundle-sheath cells are compactly arranged, the mesophyll cells have numerous intercellular spaces among them. The network of intercellular spaces is continuous from the outer surface of the bundle sheath to the large substomatal chambers, which occur in both upper and lower portions of the blade between bundles (Figs. 1, 2). We cannot corroborate the observation of Kirchanski (1975) that in *Z. mays* the extensive intercellular spaces in contact with mesophyll cells are completely sealed off from the bundle sheath by an intervening layer of mesophyll cells.

Each mesophyll cell is in direct contact with one or more sheath cells. In addition, there apparently are never more than two layers of mesophyll cells between adjacent longitudinal bundles. Using the terminology coined by Hattersley and Watson (1975), *Z. mays* has a "maximum cells distant count" of 0, no mesophyll cell being separated from the nearest bundle-sheath cell by any other chlorenchymatous cell, and a "maximum lateral cells count" of 2, only 2 mesophyll cells intervening between bundle-sheath cells of laterally (longitudinally) adjacent vascular bundles.

Distribution of the Plasmodesmata

Plasmodesmata between mesophyll cells and sheath cells, between sheath cells, and between sheath cells and vascular parenchyma cells are aggregated in primary pit-fields, which are readily detected in macerated tissues with the phase-contrast microscope (Figs. 3–6). As can be seen in Figure 3, the primary pit-fields of the outer sheath cell walls occur in groups that correspond roughly to the areas of contact between sheath cells and "arms" or lobes of the mesophyll cells. Primary pit-fields are fairly uniformly distributed in the walls between sheath cells (Fig. 4) and in those between sheath cells and vascular parenchyma cells (Figs. 4, 5). The sizes of primary pit-fields are quite variable and, correspondingly, likewise are the number of plasmodesmata per pit-field. For example, the number of plasmodesmata encountered in pit-fields of the mesophyll-sheath cell walls ranged from 34 to 103 (Fig. 15), although the actual range may be substantially greater.

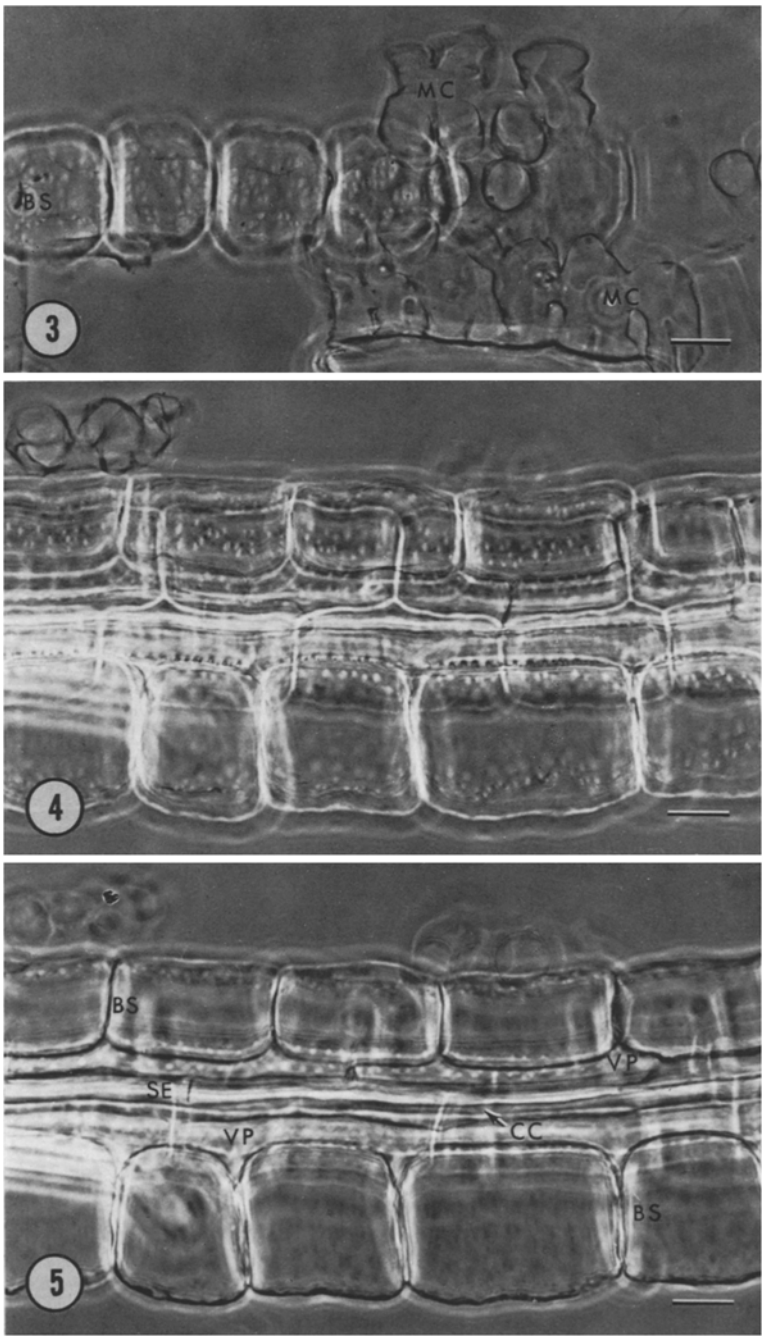
Plasmodesmata between mesophyll cells (Figs. 8, 9), between mesophyll cells and epidermal cells (Fig. 7), and between epidermal cells (Fig. 6) occur in aggregates in thickened portions of the walls, rather than in primary pit-fields. Although these thickened wall portions are not as readily detected with either light or phase contrast microscopes as the primary pit-fields, examination of a large number of thin sections, many in series, with the electron microscope, showed that they are quite common (Fig. 2).

There is no doubt that all cell types of the *Z. mays* leaf considered in this article – epidermal (excluding guard cells), mesophyll, bundle sheath, and vascular parenchyma – have numerous plasmodesmatal connections both with similar cell types (e.g., mesophyll-mesophyll) and with dissimilar, neighboring cell types (e.g., mesophyll-bundle sheath). No attempt was made to quantify the frequency of plasmodesmata between cell types, because the accuracy of any estimate of plasmodesmatal frequency would be highly problematical (see "Open Discussion" in Robards, 1976).

Structure of the Plasmodesmata

As mentioned previously, the plasmodesmata between epidermal cells occur in aggregates in thickened portions of the wall. Each unbranched plasmodesma contains a desmotubule, which appears to be tightly bound by a neck at either end of the plasmodesmatal canal (Fig. 6). Although not apparent in Figure 6, the desmotubule of each plasmodesma is in contact with the ER of both cells.

The plasmodesmata between epidermal and mesophyll cells commonly are branched on one or both sides of the thickened wall. Figure 7 shows branched and unbranched plasmodesmata in the same aggregate. As with the plasmodesmata between epidermal cells, the desmotubules of the epidermal-mesophyll cell plasmodesmata are tightly bound by a neck at either end of the canal. In addition, on the mesophyll



Figs. 3–5. Phase contrast micrographs of partial macerates of *Zea mays* leaf. *BS* bundle sheath; *CC* companion cell; *MC* mesophyll cell; *SE* sieve element; *VP* vascular parenchyma cell. All bars = 10.0 μm

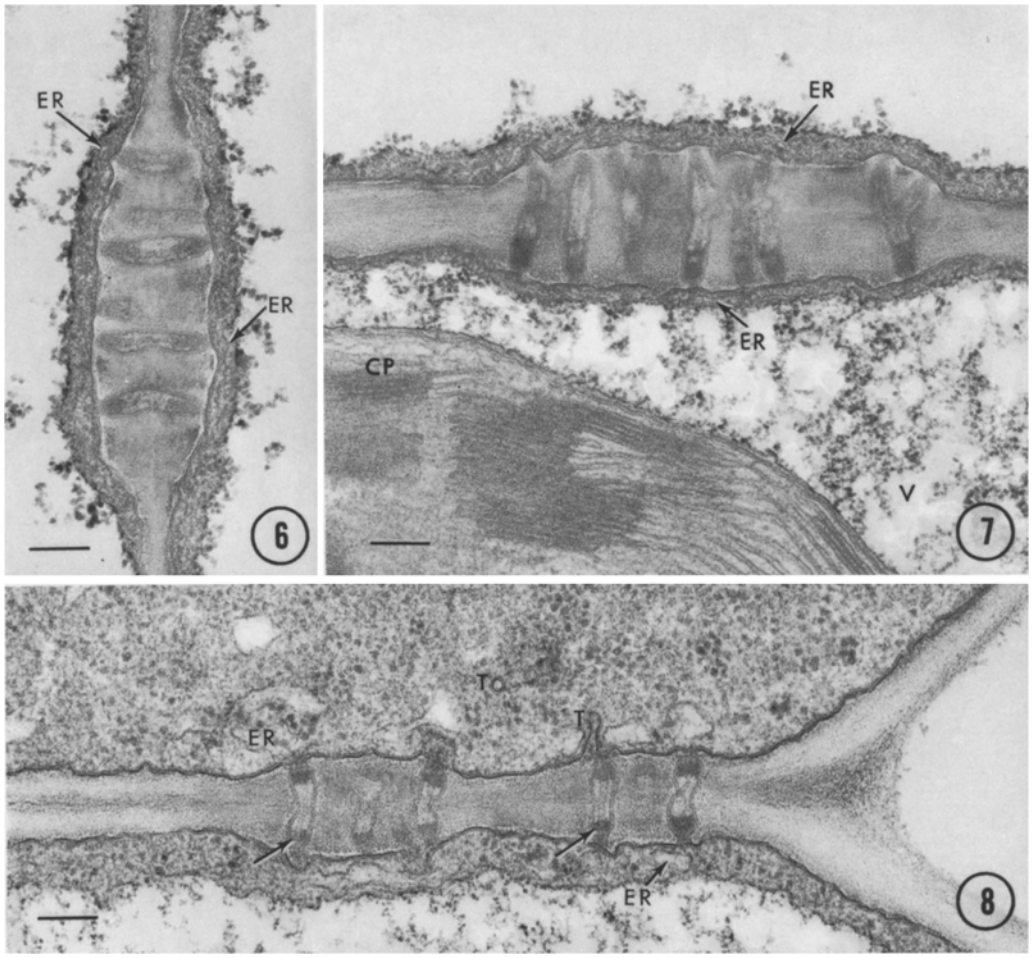
Fig. 3. Part of bundle sheath with some mesophyll cells still attached to it. Note deeply lobed condition of mesophyll cells and groups of primary pit-fields in sheath cell walls

Figs. 4 and 5. Small vein and associated bundle sheath in two planes of focus. Figure 4 focused on bundle sheath cells; Figure 5 focused on phloem in lower part of vein. Note numerous primary pit-fields in walls of bundle sheath and vascular parenchyma cells

side of the wall, the desmotubule is surrounded by an electron-opaque substance, or structure, that completely occludes the cytoplasmic annulus adjacent to the neck (Fig. 7). The desmotubule of each plasmodesma is associated with ER.

The plasmodesmata between mesophyll cells are unbranched and all are characterized by the presence of electron-dense structures near both ends of the plasmodesmatal canal (Figs. 8, 10). The structures occlude the cytoplasmic annulus where they occur and apparently they are similar to those on the mesophyll-side of the plasmodesmata between epidermal and mesophyll cells. Perhaps these structures are the ultrastructural equivalent of the hypothetical sphincters that Gunning (1976) recently suggested electron microscopists should be looking for in plasmodesmata. We shall hence forth refer to them as "sphincters", without regard to their possible function.

In median longitudinal sections of mesophyll-cell plasmodesmata (Fig. 8), the desmotubules can be seen

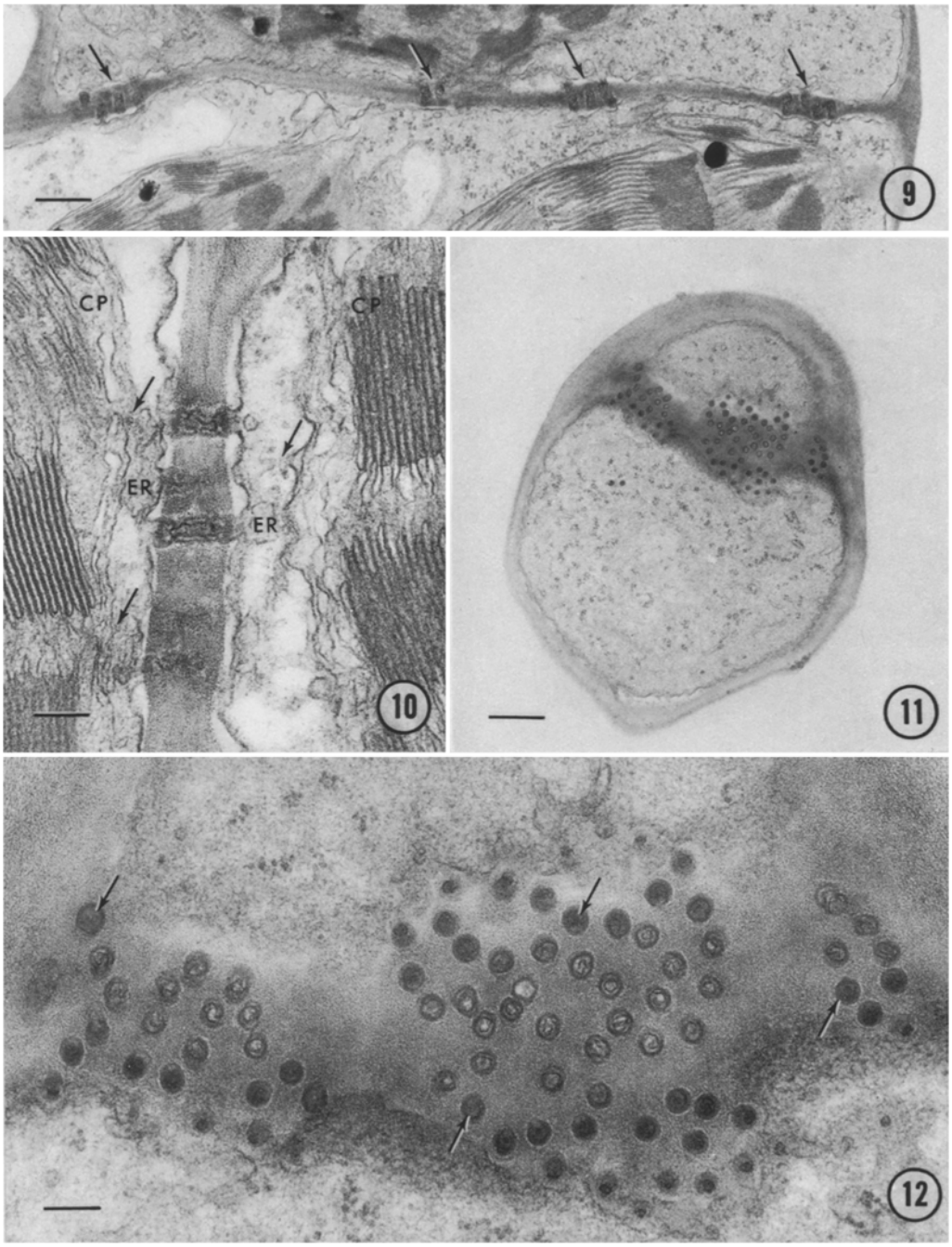


Figs. 6–8. Electron micrographs with longitudinal views of plasmodesmata in walls of *Zea mays* leaf cells. The endoplasmic reticulum associated with the plasmodesmata in Figures 6 and 7 is barely discernible in the dense cytoplasm. *CP* chloroplast; *ER* endoplasmic reticulum; *T* tubular extension of plasmalemma; *V* vacuole. **Fig. 6.** Plasmodesmata in radial wall between epidermal cells. Bar = 0.14 μm. **Fig. 7.** Plasmodesmata, some branched, in wall between epidermal cell (above) and mesophyll cell (below). Note the electron-dense substance or structures occluding the cytoplasmic annuli on the mesophyll-cell side of the wall. In some of the plasmodesmata the structures appear to consist of two parts, an outer narrow part and an inner wider part. In this article such structures are called "sphincters". Bar = 0.18 μm. **Fig. 8.** Plasmodesmata in wall between mesophyll cells. Note the sphincters near both ends of the plasmodesmata. The desmotubules are of fairly uniform width. Unlabeled arrows point to sites where the desmotubules can be seen to be continuous through the sphincters. Bar = 0.14 μm

to be continuous through the sphincters. The desmotubules of some plasmodesmata are of uniform width for their entire length (Fig. 8), while those of others are wider in the median part of the plasmodesmatal canal between sphincters (Fig. 10). This portion of the desmotubule is decidedly membranous in appearance. Figure 11 shows portions of two mesophyll cells and a glancing section through part of the wall separating them. In the higher magnification view of Figure 12, the plasmodesmata can be seen, in transverse section, at various levels of the wall. The plasmodesmata appear electron dense in the plane of the sphincters, where the narrow portions of the desmotubules are barely discernible. In median portions of the plasmodesmata, the desmotubules are relatively wide and, overall, the plasmodesmata are light in appearance.

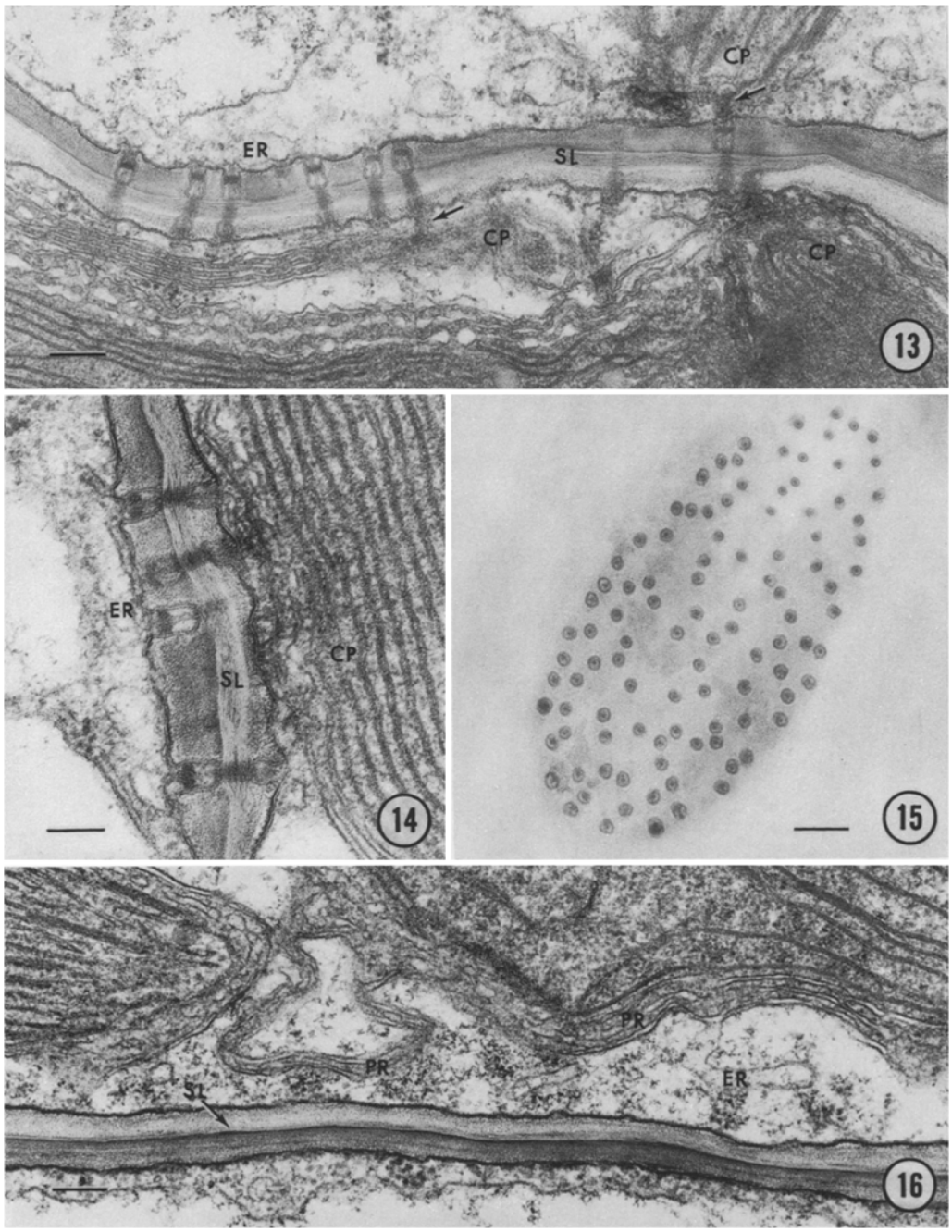
Considerable variation exists in the width of the plasmodesmatal orifices in mesophyll cell walls. Some (Fig. 8) have well developed neck constrictions, while others have no constrictions whatever. Portions of the cytoplasmic annuli of all mesophyll plasmodesmata are occluded by the sphincters. ER generally can be seen in contact with the desmotubules on both sides of the wall.

Each plasmodesma traversing the mesophyll-bundle-sheath cell wall is characterized by the presence of a sphincter on the mesophyll side of the wall and



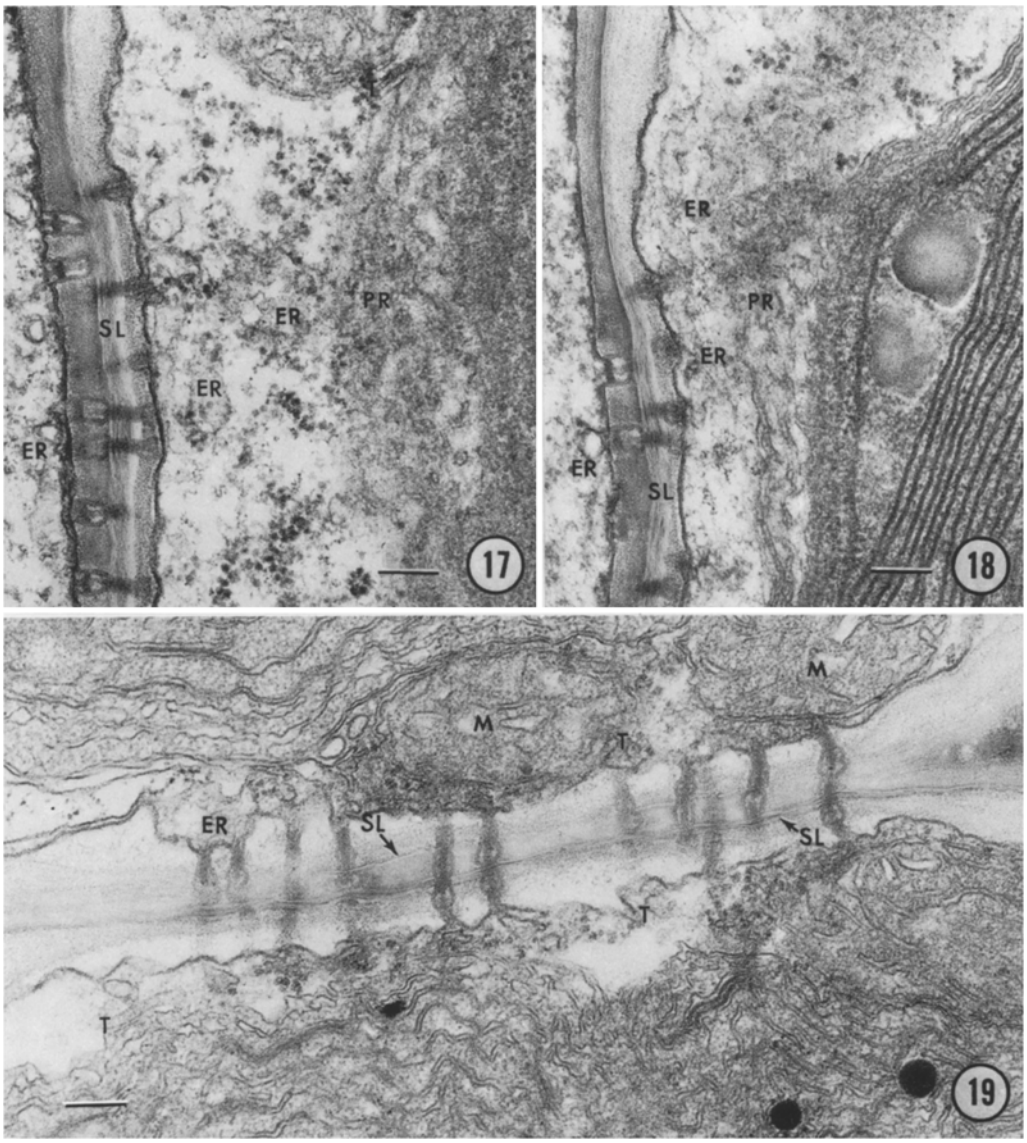
Figs. 9–12. Electron micrographs with longitudinal (Figs. 9 and 10) and transverse (Figs. 11 and 12) views of plasmodesmata in walls between mesophyll cells of the *Zea mays* leaf. *CP* chloroplast; *ER* endoplasmic reticulum. **Fig. 9.** Portions of two mesophyll cells with aggregates of plasmodesmata (arrows) in their common wall. Bar = 0.33 μm. **Fig. 10.** In the plasmodesmata shown here the desmotubule is relatively wide and membranous in appearance between sphincters. Arrows point to apparent connections between chloroplasts and endoplasmic reticulum, which is in contact with desmotubules of plasmodesmata. Bar = 0.14 μm. **Fig. 11.** Portions of two mesophyll cells and part of oblique wall between them. Bar = 0.56 μm. **Fig. 12.** Detail of Figure 11. Arrows point to the electron-dense sphincters of the plasmodesmata. As in Figure 10, the desmotubles are wider between sphincters than elsewhere. Bar = 0.15 μm

by a conspicuous constriction resulting from the presence of a wide suberin lamella next to the middle lamella on the sheath-cell side (Figs. 13, 14). In the region of the suberin lamella the plasmalemma lining the canal is closely pressed against the desmotubule at places. In near median sections of the constriction, the suberin lamella appears to be continuous through the plasmodesmatal canal (Fig. 14). The plasmodesmatal orifice on the sheath-cell side of the wall may or may not be partly constricted by a neck. Once



Figs. 13–15. Electron micrographs with longitudinal (Figs. 13 and 14) and transverse (Fig. 15) views of plasmodesmata in mesophyll-bundle sheath cell walls of the *Zea mays* leaf. *CP* chloroplast; *ER* endoplasmic reticulum; *PR* peripheral reticulum; *SL* suberin lamella. **Fig. 13.** The plasmodesmata have sphincters only on the mesophyll-cell side of such walls (above). On the sheath-cell side (below) the cytoplasmic annuli are constricted by a wide suberin lamella. Arrows point to apparent connections between chloroplasts and desmotubules of plasmodesmata. Bar = 0.16 μm. **Fig. 14.** Detail of plasmodesmata in wall between mesophyll cell (left) and sheath cell (right). Again, note that sphincters occur only on the mesophyll-cell side of such connections. The suberin lamella on the sheath-cell side has a distinct multilayered appearance, resembling the suberin lamella of the endodermal cell walls of the root (Karas and McCully, 1973). Bar = 0.11 μm. **Fig. 15.** Surface view of primary pit-field on sheath-cell side of wall with an aggregate of 103 plasmodesmata. Bar = 0.32 μm

Fig. 16. Portions of sheath (above) and mesophyll (below) cells, with a segment of wall lacking plasmodesmata between them. In such regions of the wall the suberin lamella is relatively narrow. Blebbing of the peripheral reticulum of the sheath cell chloroplasts as seen here (left) was fairly common. A segment of endoplasmic reticulum can be seen in continuity with the outer chloroplast membrane to the right. Bar = 0.11 μm



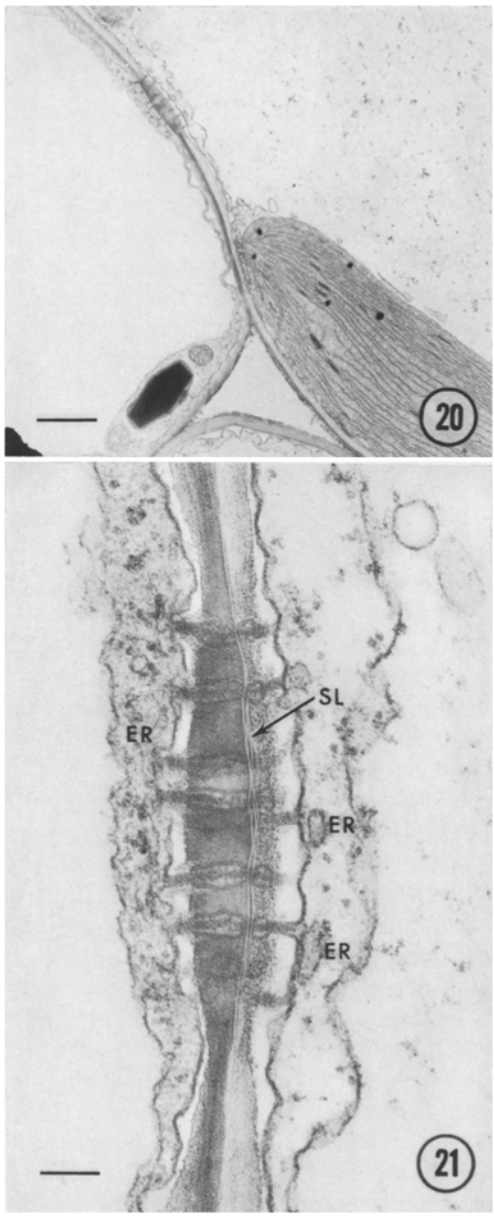
Figs. 17–19. Electron micrographs of plasmodesmata in leaf of *Zea mays*. *ER* endoplasmic reticulum; *M* mitochondrion; *PR* peripheral reticulum; *SL* suberin lamella; *T* tubular extension of plasmalemma. **Figs. 17, 18.** Show glancing sections through peripheral reticulum and envelope of bundle sheath chloroplasts. Note apparent continuity of chloroplasts with endoplasmic reticulum, which is continuous with desmotubules of the plasmodesmata. Sphincters are conspicuous components of plasmodesmata on mesophyll-cell side of wall. Figure 17 bar = 0.11 μm; Figure 18 bar = 0.11 μm. **Fig. 19.** Longitudinal view of plasmodesmata in radial wall between contiguous sheath cells. Suberin lamellae occur on both sides of the compound middle lamella, but they are not always apparent when sectioned obliquely. Bar = 0.15 μm

again, ER commonly can be seen in contact with the desmotubules on both sides of the wall.

The suberin lamella of the sheath cell wall is wider in the region of the plasmodesmata between mesophyll cells and sheath cells than elsewhere (e.g., compare Figs. 13, 14 with Fig. 16). The lamella, which stains with sudan IV, is continuous in the outer tangential and radial walls of all sheath cells, including those of the transverse veins.

Two suberin lamellae occur in the radial walls between adjacent sheath cells, one on each side of the compound middle lamella. The plasmodesmata between sheath cells, therefore, have two constrictions associated with suberin lamellae (Fig. 19). As a result, the portion of the plasmodesmatal canal in the region of the compound middle lamella often resembles a median nodule or cavity. Both ends of the sheath-cell plasmodesmata have neck constrictions.

The final plasmodesmata to be considered in this article are those between sheath cells and vascular



Figs. 20 and 21. Electron micrographs of portions of bundle sheath (right) and vascular parenchyma (left) cells with group of plasmodesmata in their common wall. *ER* endoplasmic reticulum; *SL* suberin lamella. **Fig. 20.** Plastid with electron-dense protein crystalloid can be seen, below, in the vascular parenchyma cell; opposite it in the sheath cell is an "agranal" plastid typical of the sheath cells in *Z. mays*. Bar = 0.86 μm. **Fig. 21.** Detail of group of plasmodesmata from Figure 20 showing suberin lamella on sheath-cell side of wall. Bar = 0.15 μm

parenchyma cells, which are characterized by the presence of plastids with electron-dense protein crystalloids (Fig. 20). The vascular parenchyma cells often are in contact with both tracheary elements and phloem cells; hence, the general designation given them.

The inner tangential walls of the bundle-sheath cells, including those in contact with vascular parenchyma cells, apparently are not suberized throughout. The only portions of the wall consistently and conspicuously suberized are those traversed by plasmodesmata uniting the protoplasts of bundle-sheath cells with vascular parenchyma cells (Fig. 21). These plasmodesmata are constricted by the suberin lamella and have neck constrictions on both sheath- and vascular-parenchyma-cell sides of the wall.

Chloroplast-Plasmodesmata Associations

The chloroplasts of both mesophyll cells and bundle-sheath cells of C_4 plants, including *Z. mays,* typically contain a peripheral reticulum (PR) of anastomosing tubules, which some workers (e.g. Slack et al., 1969; Gracen et al., 1972; Chapman et al., 1975) have suggested may play a role in the rapid transfer of substances into and out of the chloroplasts and from mesophyll to bundle-sheath cells. It has further been suggested that physical connections may exist between chloroplasts by way of the plasmodesmata in the mesophyll-bundle-sheath cell walls (Slack et al., 1969). For this reason, much time was spent during the present study in search of evidence of the presence of connections between chloroplasts and plasmodesmata in mesophyll and bundle-sheath cells.

Numerous cases were encountered in which the outer membrane of the chloroplast envelope apparently was connected with the desmotubule of a plasmodesma by means of a short segment of endoplasmic reticulum. Such apparent connections were found linking chloroplasts with plasmodesmata in walls between mesophyll cells (Fig. 10), between mesophyll cells and sheath cells (Figs. 13, 17, 18), and between sheath cells. Very often the chloroplasts—especially those of sheath cells—were so close to the plasmodesmata that it was impossible to determine whether any direct connections existed between plastids and plasmodesmata (Fig. 14).

Most of the mesophyll-cell chloroplasts do not lie next to plasmodesmata in the mesophyll-sheath-cell walls. Rather, they are more or less uniformly distributed in the parietal layer of cytoplasm along all walls of the mesophyll cells (Fig. 2). Direct connections occasionally were encountered between ER and plastid envelope in portions of mesophyll and sheath (Fig. 16) cells not contiguous to plasmodesmata.

Examination of 0.5 μm sections with the high-voltage electron microscope failed to confirm the presence of connections between plastids and plasmodesmata by way of the ER. This is because the ER was not discernible in such thick sections, although

tubular extensions of the plasmalemma were clearly visible within them (Evert et al., 1977). Efforts are underway to solve this problem through improved staining techniques. However, even in thin sections the ER was not very conspicuous (Figs. 8, 10, 13, 14), whereas tubular extensions of the plasmalemma were quite obvious in solely osmium tetroxide-fixed tissues (Fig. 8).

Discussion

In *Zea mays*, as in other NADP-malic enzyme species, atmospheric CO_2 is initially fixed by phosphoenolpyruvate carboxylase in the mesophyll cytoplasm to form oxaloacetic acid, which is then reduced to malate in the mesophyll chloroplasts by NADP-malate dehydrogenase (Chollet and Ogren, 1973; Hatch et al., 1975; Ku and Edwards, 1975). The malate is transported to the chloroplasts of the bundle-sheath cells where it is decarboxylated by NADP-malic enzyme to form pyruvate and CO_2. The CO_2 then is refixed by ribulose-1,5-diphosphate carboxylase to form 3-phosphoglyceric acid as it is incorporated into the reductive pentose-phosphate pathway of the bundle-sheath cells. The pyruvate returns to the mesophyll cells to be regenerated to phosphoenolpyruvate.

Z. mays and other species of Panicoideae with high NADP-malic enzyme activity and grana-poor bundle-sheath chloroplasts exhibit little or no photosystem II activity in their bundle-sheath cells (Anderson et al., 1971; Ku et al., 1974; Osmond, 1974; Usada et al., 1975). It has been proposed that in these species NADPH generated by NADP-malic enzyme during the production of CO_2 provides the reducing power for the fixation of CO_2 in the bundle-sheath cells (Guiterrez et al., 1974; Farineau, 1975a, b; Ku and Edwards, 1975), but that some phosphoglyceric acid – perhaps as much as half the phosphoglyceric acid formed in the bundle-sheath cells (Hatch and Kagawa, 1976) – may have to be shuttled to the mesophyll cells for reduction to dihydroxyacetone phosphate, which probably is transported back to the sheath cells (Karpilov and Bil', 1976). Thus, in *Z. mays* and other NADP-malic enzyme species intermediate products of photosynthesis are transported in both directions between mesophyll and bundle-sheath cells: malate and probably dihydroxyacetone phosphate from mesophyll to sheath cells, and pyruvate and probably phosphoglyceric acid in the opposite direction.

Calculations of the amounts and rates of movement of C_4 acids between chloroplasts of mesophyll and bundle-sheath cells during photosynthesis in C_4 plants have led some workers to suggest that the rapid movement of C_4 acids between chloroplasts of the two cell types may be accomplished either entirely by diffusive processes (Osmond, 1971; Black, 1973; Osmond and Smith, 1976) or by volume flow (Gunning and Robards, 1976a) via the plasmodesmata. Others (Bil' et al., 1976; Karpilov and Bil', 1976) have suggested that the transport of intermediates of photosynthesis in C_4 plants is an active process involving large numbers of cytoplasmic vesicles that carry the metabolites between chloroplasts and plasmalemma.

Implication of plasmodesmata in the intercellular transport of photosynthetic intermediates in C_4 plants results in part from their great frequency in the mesophyll-bundle-sheath cell walls (Olesen, 1975; Osmond and Smith, 1976) and partly from the presence of the suberin lamella, which presumably precludes apoplastic movement of metabolites between mesophyll and bundle-sheath cells in C_4 monocotyledons, in the sheath-cell wall (Läuchli, 1976).

Plasmolytic studies in our laboratories (to be reported in detail in a subsequent article) indicate that the suberin lamella of the bundle-sheath cells in leaves of *Z. mays* is fairly impermeable to water. Undamaged bundle-sheath cells (i.e. cells without ruptured walls) showed no evidence of plasmolysis with the light microscope after more than 2 h in a 1.5-M sucrose solution (osmotic potential, −66.7 bars). By comparison, undamaged, contiguous mesophyll cells plasmolyzed in 0.3 to 0.4 molar sucrose solutions (osmotic potentials, −8.24 to −11.26 bars) in a matter of minutes. In another study, Lush and Evans (1974) found plasmolysis difficult to detect in bundle-sheath cells of *Paspalum dilatatum* in less than 31% sucrose solutions. These results support those of other workers concerned with the effects of water stress on the structure of mesophyll and bundle-sheath cells in *Z. mays* and *Sorghum bicolor* (Giles et al., 1974; Giles et al., 1976). In both species, bundle-sheath cells exhibited greater resistance than mesophyll cells to damage under water stress.

Evidence from carbon-isotope fractionation studies indicates that the bundle-sheath layer of C_4 plants is also impermeable to CO_2 (Troughton, 1972; Whelan et al., 1973). This may explain in part the apparent lack of photorespiratory CO_2 loss from C_4 plants (Chollet and Ogren, 1975).

Suberin lamellae also occur in the mestome sheath walls of C_3 monocotyledons (O'Brien and Carr, 1970; Miyake and Maeda, 1976). (Curiously, suberin lamellae are lacking in bundle sheath walls of C_4 dicotyledons; Laetsch, 1971.) The suberin lamella in wheat, a C_3 plant, has been demonstrated to inhibit the passage of dyes carried in the transpiration stream beyond the mestome sheath (O'Brien and Carr, 1970).

It seems quite certain, therefore, that the transport of metabolites between mesophyll and mestome-

sheath cells of C_3 monocotyledons and mesophyll and bundle-sheath cells of C_4 monocotyledons, such as *Z. mays,* follows a symplastic pathway via the plasmodesmata. Considerable discussion has taken place as to whether both the cytoplasmic annulus and the desmotubule or the desmotubule alone provides the transport pathway across the wall (Olesen, 1975; Gunning, 1976; Gunning and Robards, 1976a, b; Hattersley et al., 1976; Osmond and Smith, 1976). The ultrastructural evidence indicates that the suberin lamella of the bundle-sheath wall of C_4 monocotyledons and of the mestome wall of C_3 monocotyledons constricts the cytoplasmic annulus (O'Brien and Carr, 1970) probably leaving the desmotubule as the only open pathway between the two layers of chlorenchymatous cells. This condition is exemplified by the plasmodesmata traversing the mesophyll-bundle-sheath walls of *Z. mays*. In addition, in *Z. mays* suberin lamella constrictions occur in plasmodesmata between sheath cells and vascular parenchyma cells, while "double" suberin lamella constrictions are found in plasmodesmata between contiguous sheath cells. With the exception of many of the plasmodesmata between mesophyll cells and between mesophyll and bundle-sheath cells – plasmodesmata the cytoplasmic annuli of which are partly occluded by sphincters – neck constrictions were encountered in all plasmodesmata examined during the present study. Thus, it appears that the desmotubule may be the only open plasmodesmatal pathway between all cells of the *Z. mays* leaf considered in this article.

Even with the desmotubules as the only pathways for the transport of photosynthetic metabolites between mesophyll and bundle-sheath cells, such transport apparently could occur efficiently by diffusion alone, provided that sufficiently large concentration gradients are maintained by carboxylation and decarboxylation (Osmond and Smith, 1976). Gunning and Robards (1976a) and Osmond and Smith (1976) have noted that since the desmotubule is attached to the ER one must take into account solute movement across the ER membrane at the time of loading and unloading of the ER. During the present study evidence was found for the presence of direct connections between chloroplasts and desmotubules via the ER in both mesophyll and bundle-sheath cells. Such connections would provide a direct pathway for photosynthetic intermediates to move from mesophyll chloroplast to sheath chloroplast, or vice versa, via the intracisternal space of the ER. It is not difficult to envisage a volume flow between chloroplasts within the ER and desmotubules.

O'Brien and Carr (1970) noted the frequent appearance of mitochondria in the pit cavities of the mestome cells of the wheat leaf, and suggested that the mitochondria may provide energy for solute transfer at the pit-fields. Mitochondria often were observed in the cytoplasm contiguous to primary pit-fields of the mesophyll-bundle sheath wall examined during the present study. More often, however, no mitochondria were found in the near vicinity of the pit-fields.

The conclusion of Karpilov and Bil' (1976) that cytoplasmic vesicles serve as "temporary channels or transport structures" between chloroplast and plasmalemma results in part from their observations that ER is almost totally lacking in chlorenchymatous leaf cells of C_4 plants. ER was fairly abundant in all parenchymatic elements of the *Z. mays* leaves examined during the present study, although it was not a conspicuous component of the protoplast. Preservation of the protoplasts in the published electron micrographs of Karpilov and Bil' appears to be less than satisfactory, indicating that many of their vesicles may have been derived from poorly preserved ER.

Inasmuch as photosynthetic metabolites move in both directions between mesophyll and bundle-sheath cells of C_4 plants, considerable discussion has taken place over the feasibility of bidirectional transport through the plasmodesmata between these two cell types (Gunning and Robards, 1976a, b; Hattersley et al., 1976; Osmond and Smith, 1976). As pointed out by Gunning and Robards (1976a) some plasmodesmata might operate in one direction and others in the opposite direction, although no data are available either to prove or disprove this possibility.On the other hand, if diffusive processes alone are involved, bidirectional diffusive movement through a single plasmodesma should be able to occur without difficulty, provided the distance is relatively short.

Difficulties to bidirectional transport seemingly arise only if pressure flows take place simultaneously in opposite directions across the same wall (Gunning and Robards, 1976a). If both desmotubule and cytoplasmic annulus were to serve as transport channels, one could envisage flows in opposite directions through two independent transport channels of a single plasmodesma (Gunning, 1976). Occlusion of the cytoplasmic annulus by the sphincter and its constriction by the suberin lamella seemingly precludes this possibility between mesophyll and bundle-sheath cells of *Z. mays*. On the other hand, the presence of the sphincters in the plasmodesmata of the mesophyll-bundle-sheath cell walls may provide an answer to the dilemma of bidirectional transport between these cell layers in the *Z. mays* leaf. It is tempting to suggest that the sphincters may represent plasmodesmatal valves capable of controlling the directions and rates of transport of substances throught the desmotubules. Cytochemical studies may prove useful in determining the nature of the sphincters and their possible role

in the regulation of metabolite transport through the desmotubules.

In addition to the problem of bidirectional flow of photosynthetic intermediates between mesophyll and bundle-sheath cells in C_4 plants is that of transpirational water movement and its effect on the movement of the photosynthetic intermediates. Kuo et al. (1974) have suggested that in wheat, a C_3 plant, the major water fluxes take place from different bundles and in different parts of the mestome sheath from the sugar fluxes. Kuo (see discussion in Osmond and Smith, 1976) since has suggested that the transverse veins, which unlike the longitudinal veins lack a suberin lamella in the wheat leaf, might provide a route for water to enter the mesophyll without interfering with the "trans-bundle sheath fluxes" in the longitudinal veins.

Whereas the mestome sheath cells of the longitudinal veins of wheat are completely encased in a suberin lamella (O'Brien and Carr, 1970), the bundle-sheath cells of *Z. mays* apparently have only their outer tangential and radial walls completely suberized. The inner tangential walls exhibit a suberin lamella consistently only at the sites of plasmodesmata. In addition, the bundle-sheath cells of the transverse veins of the *Z. mays* leaf have only their outer tangential and radial walls suberized opposite the xylem. Hence, in the *Z. mays* leaf the apoplast between the xylem and the mesophyll is not completely interrupted by a suberin lamella. Water entering the walls of parenchymatic elements or of sheath cells bordering the xylem can move to the mesophyll cell walls via the compound middle lamella between suberin lamellae in the radial walls of the sheath cells. This proposed pathway is in agreement with studies that point to the cell walls as the major pathways for transpirational water from the xylem to the evaporating surfaces of mesophyll cells (Crowdy and Tanton, 1970; Weatherley, 1970; Läuchli, 1976). In the *Z. mays* leaf movement of the photosynthetic intermediates between mesophyll and bundle-sheath cells apparently is restricted largely or entirely to a symplastic pathway, while transpirational water movement apparently is restricted largely or entirely to an apoplastic pathway.

Finally, a word about the tubular extensions of the plasmalemma reported earlier in leaf cells of *Z. mays* (Evert et al., 1977). It is apparent that these tubules, which are numerous in both mesophyll and bundle-sheath cells, greatly extend the symplast-apoplast interface. Arising near the plasmodesmata, many of the tubules extend toward the chloroplasts and lie in close proximity to them. The tubules provide a means of enlarging the volume of the apoplastic pathway, the pathway for the movement of both water and solutes necessary for metabolic activities of the cell. The close procimity of the tubules to the ER and plasmodesmata would provide a ready supply of water necessary for a volume flow of photosynthetic intermediates between mesophyll and bundle-sheath cells via the ER and desmotubules.

O'Brien and Carr (1970) have suggested that transfer cells may be absent from grass leaves because of the presence of the suberin lamella in the walls of the bundle-sheath cells; that, if the bulk of solute transfer takes place through plasmodesmata rather than across free space, transfer cells would be unnecessary. The absence of transfer cells in the leaves of *Z. mays* might be explained by the presence of the tubular extensions of the plasmalemma, which greatly extend the surface of the plasmalemma without the presence of wall ingrowths in mesophyll, bundle-sheath, and vascular-parenchyma cells (Evert et al., 1977).

This work was initiated in 1975, while the first author was an Alexander von Humboldt-Stiftung Awardee at the Forstbotanisches Institut Göttingen. Thanks are extended to Roswitha Burchardt, Susan E. Eichhorn, Damian S. Neuberger and Lynn Smook for their technical assistance. This research was supported in part by National Science Foundation grant DEB76-10585 to R.F.E. The AEI, EM-7 high-voltage electron microscope is supported by National Institutes of Health research grant RR00570 from the Biotechnology Resources Branch, Division of Research Resources.

References

Anderson, J.M., Boardman, N.K., Spencer, D.: Phosphorylation by intact bundle sheath chloroplasts from maize. Biochim. Biophys. Acta **245**, 253–258 (1971)

Bil', K.Ya., Belobrodskaya, L.K., Karpilov, Yu.S.: Localization of ATPases in cellular structures of assimilating tissues in amaranth leaves. Dokl. Akad. Nauk SSSR (Engl. transln.) Bot. Sci. **226**, 16–19 (1976)

Black, C.C., Jr.: Photosynthetic carbon fixation in relation to net CO_2 uptake. Ann. Rev. Plant Physiol. **24**, 253–286 (1973)

Chapman, E.A., Bain, J.M., Gove, D.W.: Mitochondria and chloroplast peripheral reticulum in the C_4 plants *Amaranthus edulis* and *Atriplex spongiosa*. Aust. J. Plant Physiol. **2**, 207–223 (1975)

Chollet, R., Ogren, W.L.: Photosynthetic carbon metabolism in isolated maize bundle sheath strands. Plant Physiol. **51**, 787–792 (1973)

Chollet, R., Ogren, W.L.: Regulation of photorespiration in C_3 and C_4 species. Bot. Rev. **41**, 137–179 (1975)

Crowdy, S.H., Tanton, T.W.: Water pathways in higher plants. I. Free space in wheat leaves. J. exp. Bot. **21**, 102–111 (1970)

Esau, K.: Ontogeny of the vascular bundle in *Zea mays*. Hilgardia **15**, 327–368 (1943)

Evert, R.F., Eschrich, W., Neuberger, D.S., Eichhorn, S.E.: Tubular extensions of the plasmalemma in leaf cells of *Zea mays* L. Planta 1977

Farineau, J.: Photoassimilation of CO_2 by isolated bundle-sheath strands of *Zea mays*. I. Stimulation of CO_2 assimilation by adding various intermediates of the phtosynthetic cycle; evidence for a deficient photosystem II activity. Physiol. Plantarum **33**, 300–309 (1975a)

Farineau, J.: Photoassimilation of CO_2 by isolated bundle-sheath strands of *Zea mays*. II. Role of malate as a source of CO_2 and reducing power for photosynthetic activity of isolated bundle-sheath cells of *Zea mays*. Physiol. Plantarum **33**, 310–315 (1975b)

Geiger, D.R.: Phloem loading in source leaves. In: Transport and transfer processes in plants, pp. 167–183, Wardlaw, I.F., Passioura, J.B., eds. New York-San Francisco-London: Academic Press 1976

Giles, K.L., Beardsell, M.F., Cohen, D.: Cellular and ultrastructural changes in mesophyll and bundle sheath cells of maize in response to water stress. Plant Physiol. **54**, 208–212 (1974)

Giles, K., Cohen, D., Beardsell, M.F.: Effects of water stress on the ultrastructure of leaf cells of *Sorghum bicolor*. Plant Physiol. **57**, 11–14 (1976)

Gracen, V.E., Jr., Hilliard, J.H., Brown, R.H., West, S.H.: Peripheral reticulum in chloroplasts of plants differing in CO_2 fixation pathways and photorespiration. Planta (Berl.) **107**, 189–204 (1972)

Gunning, B.E.S.: Introduction to plasmodesmata. In: Intercellular communication in plants: Studies on plasmodesmata, pp. 1–13, Gunning, B.E.S., Robards, A.W., eds. Berlin-Heidelberg-New York: Springer 1976

Gunning, B.E.S., Robards, A.W.: Plasmodesmata: current knowledge and outstanding problems. In: Intercellular communications in plants: Studies on plasmodesmata, pp. 297–311, Gunning, B.E.S., Robards, A.W., eds. Berlin-Heidelberg-New York: Springer 1976 (a)

Gunning, B.E.S., Robards, A.W.: Plasmodesmata and symplastic transport. In: Transport and transfer processes in plants, pp. 15–41, Wardlaw, I.F., Passioura, J.B., eds. New York-San Francisco-London: Academic Press 1976 (b)

Gutierrez, M., Gracen, V.E., Edwards, G.E.: Biochemical and cytological relationships in C_4 plants. Planta (Berl.) **119**, 279–300 (1974)

Hatch, M.D., Kagawa, T.: Photosynthetic activities of isolated bundle sheath cells in relation to differing mechanisms of C_4 pathway photosynthesis. Arch. Biochem. Biophys. **175**, 39–53 (1976)

Hatch, M.D., Kagawa, T., Craig, S.: Subdivision of C_4-pathway species based on differing C_4 acid decarboxylating systems and ultrastructural features. Aust. J. Plant Physiol. **2**, 111–128 (1975)

Hattersley, P.W., Watson, L.: Anatomical parameters for predicting photosynthetic pathways of grass leaves: the "maximum lateral cell count" and the "maximum cells distant count". Phytomorphology **25**, 325–333 (1975)

Hattersley, P.W., Watson, L., Osmond, C.B.: Metabolite transport in leaves of C_4 plants: specification and speculation. In: Transport and transfer processes in plants, pp. 191–201, Wardlaw, I.F., Passioura, J.B., eds. New York-San Francisco-London: Academic Press 1976

Heyser, W., Leonard, O., Heyser, R., Fritz, E., Eschrich, W.: The influence of light, darkness, and the lack of CO_2 on phloem translocation in detached maize leaves. Planta (Berl.) **122**, 143–154 (1975)

Heyser, W., Heyser, R., Eschrich, W., Leonard, O.A., Rautenberg, M.: The influence of externally applied organic substances on phloem translocation in detached maize leaves. Planta (Berl.) **132**, 269–277 (1976)

Karas, I., McCully, M.E.: Further studies of the histology of lateral root development in *Zea mays*. Protoplasma **77**, 243–269 (1973)

Karpilov, Yu.S., Bil', K.Ya.: Transport of intermediate photosynthesis products through the cytoplasm of cells assimilating tissues in C_4 plants. Dokl. Akad. Nauk SSSR (Engl. transln.) Bot. Sci. **226**, 24–27 (1976)

Kirchanski, S.J.: The ultrastructural development of the dimorphic plastids of *Zea mays* L. Amer. J. Bot. **62**, 695–705 (1975)

Ku, S.B., Edwards, G.E.: Photosynthesis in mesophyll protoplasts and bundle sheath cells of various types of C_4 plants. IV. Enzymes of respiratory metabolism and energy utilizing enzymes of photosynthetic pathways. Z. Pflanzenphysiol. **77**, 16–32 (1975)

Ku, S.B., Gutierrez, M., Kanai, R., Edwards, G.E.: Photosynthesis in mesophyll protoplasts and bundle sheath cells of various types of C_4 plants. II. Chlorophyll and Hill reaction studies. Z. Pflanzenphysiol. **72**, 320–337 (1974)

Kuo, J., O'Brien, T.P., Canny, M.J.: Pit-field distribution, plasmodesmatal frequency, and assimilate flux in the mestome sheath cells of wheat leaves. Planta (Berl.) **121**, 97–118 (1974)

Laetsch, W.M.: Chloroplast structural relationships in leaves of C_4 plants. In: Photosynthesis and photorespiration, pp. 323–349, Hatch, M.D., Osmond, C.B., Slatyer, R.O., eds. New York: Wiley-Interscience 1971

Läuchli, A.: Apoplastic transport in tissues. In: Transport in plants, pp. 3–34, Lüttge, U., Pitman, M.G., eds. Berlin-Heidelberg-New York: Springer 1976

Lush, W.M., Evans, L.T.: Translocation of photosynthetic assimilate from grass leaves, as influenced by environment and species. Aust. J. Plant Physiol. **1**, 417–431 (1974)

Metcalfe, C.R.: Anatomy of the monocotyledons. I. Gramineae. Oxford: Clarendon Press 1960

Miyake, H., Maeda, E.: Development of bundle sheath chloroplasts in rice seedlings. Canad. J. Bot. **54**, 556–565 (1976)

O'Brien, T.P., Carr, D.J.: A suberized layer in the cell walls of the bundle sheath of grasses. Aust. J. biol. Sci. **23**, 275–287 (1970)

Olesen, P.: Plasmodesmata between mesophyll and bundle sheath cells in relation to the exchange of C_4-acids. Planta (Berl.) **123**, 199–202 (1975)

Osmond, C.B.: Metabolite transport in C_4 photosynthesis. Aust. J. biol. Sci. **24**, 159–163 (1971)

Osmond, C.B.: Carbon reduction and photosystem II deficiency in leaves of C_4 plants. Aust. J. Plant Physiol. **1**, 41–50 (1974)

Osmond, C.B., Smith, F.A.: Symplastic transport of metabolites during C_4-photosynthesis. In: Intercellular communication in plants: Studies on plasmodesmata, pp. 229–240, Gunning, B.E.S., Robards, A.W., eds. Berlin-Heidelberg-New York: Springer 1976

Robards, A.W.: Plasmodesmata in higher plants. In: Intercellular communication in plants: Studies on plasmodesmata, pp. 15–57, Gunning, B.E.S., Robards, A.W., eds. Berlin-Heidelberg-New York: Springer 1976

Slack, C.R., Hatch, M.D., Goodchild, D.J.: Distribution of enzymes in mesophyll and parenchyma-sheath chloroplasts of maize leaves in relation to the C_4-dicarboxylic acid pathway of photosynthesis. Biochem. J. **114**, 489–498 (1969)

Troughton, J.H.: Carbon isotope fractionation by plants. In: Proc. VIII Int. Radiocarbon Dating Conf., held at Lower Hutt, N.Z., 18–25 October; organized by the Roy. Soc. N.Z.; compiled by T.A. Rafter, T. Grant-Taylor, vol. 2, pp. 420–438 (1972)

Usuda, H., Kanai, R., Miyachi, S.: Carbon dioxide assimilation and photosystem II deficiency in bundle sheath strands isolated from C_4 plants. Plant Cell Physiol. **16**, 485–494 (1975)

Weatherley, P.E.: Some aspects of water relations. Adv. Bot. Res. **3**, 171–206 (1970)

Whelan, T., Sackett, W.M., Benedict, C.R.: Enzymatic fractionation of carbon isotopes by phosphoenolpyruvate carboxylase from C_4 plants. Plant Physiol. **51**, 1051–1054 (1973)

Received 18 April; accepted 4 May 1977